



# Prevalence of *Dipylidium caninum* in Stray and Pet Dogs in Siddharthanagar Municipality, Rupandehi, Nepal

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## Abstract

**Introduction:** *Dipylidium caninum* (double-pored tapeworm) is a common tapeworm found in the small intestines of dogs, cats, foxes, and occasionally humans. It is a zoonotic parasite that causes dipylidiasis, commonly known as dog tapeworm, which is acquired by ingesting infected fleas. The major goal of this study is to determine the prevalence of *D. caninum* in stray and pet dogs in Siddharthanagar municipality, along with its various risk factors (age, sex, breed, deworming status, and flea infestation).

**Methods:** This cross-sectional study was carried out from June to August 2021. A total of 200 fecal samples (100 stray and 100 pet) were collected purposively from different wards of Siddharthanagar municipality and examined for the presence of proglottids and eggs of *D. caninum*.

**Results:** The overall prevalence was found to be 12.5%, with a significantly higher prevalence in stray dogs (18%) than in pet dogs (7%) ( $P < 0.05$ ). Males (14.15%) showed a higher prevalence than females (10.35%), but the difference was not statistically significant ( $P > 0.05$ ). Similarly, an insignificant difference was observed between dogs aged less than one year (8.0%) and dogs older than one year (15.20%) ( $P > 0.05$ ). Based on the deworming status, the prevalence was higher in non-dewormed dogs (13.87%) than in dewormed dogs (3.70%), but the difference was found to be statistically insignificant ( $P > 0.05$ ). Similarly, significance was found in the flea infestation ( $P < 0.05$ ), with a higher prevalence in flea-infested dogs (20.56%) than in flea-non-infested dogs (3.22%). Also, only 13.5% of dog owners surveyed were aware of canine helminth zoonoses.

**Conclusion:** This study shows a comparatively higher prevalence of *D. caninum* in stray, non-dewormed, and flea-infested dogs. An awareness program to educate the local community on canine zoonoses, appropriate anti-helminthic treatment, and control of the street dog population might ultimately reduce the prevalence of gastrointestinal helminths in Siddharthanagar municipality.

**Keywords:** Dipylidiasis, Zoonoses, Deworming, Proglottids, Helminths

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## Introduction

### Background

The domestic dog belongs to the Canidae family, consisting of 35 related species that diverged within the last ten million years. Dogs (*Canis familiaris*) are the first animals to be domesticated by humans (1) and have been human companions all over the world. Relationships between pet dogs and humans are both very widespread and very intense, often leading to strong attachments between owners or caregivers and animals and the treatment of these dogs as family members or even children (2). More than 60 zoonotic diseases are associated with dogs, most of which are helminth infections (3,4). Toxoplasmosis, leishmaniasis, giardiasis, echinococcosis, dirofilariasis, and toxocarosis are described from the animal, as well as from the human host perspective, with an emphasis on parasite life cycle, transmission, pathogenicity, prevention, and identification of knowledge gaps related to dogs (5). The intimate contact between humans and dogs plays an active role in public health as they serve as reservoirs and transmitters of diseases (6).

*Dipylidium caninum* (double-pored tapeworm) is a common tapeworm occurring in the small intestines of dogs, cats, foxes, and occasionally men (7). It is a zoonotic parasite causing a disease named dipylidiasis, commonly called dog tapeworm, acquired by ingesting infected fleas (8). In the life cycle of *D. caninum*, a dog consumes a flea that is infected with larvae or cysticercoid. This process results in proglottids in the animal's feces, which are then ingested by flea larvae (9). The proglottids disintegrate in the environment, releasing egg packets that occasionally appear free in the feces (10,11). The gravid proglottids are elongated, oval-shaped with a cucumber seed-like appearance, and contain numerous egg packets, each having 5-30 eggs (12). The intermediate host (most often larval stages of the dog or cat flea (*Ctenocephalides* spp.)) ingests egg packets. The larval flea releases the oncosphere into its intestine, where it penetrates the intestinal wall, invades the insect's hemocoel (body cavity), and develops into a cysticercoid (13). Humans are accidental hosts who acquire the infection by ingesting infected dog or cat fleas (14,15).



### Statement of Problem

Dogs are reservoirs of many zoonotic diseases (16). Although dogs play important roles in the lives of such families, they serve either as definitive or reservoir hosts for many zoonotic parasites, posing major public health, economic, and social problems, particularly in developing countries where the movement and management of dogs are not commonly controlled (17). Dogs are also the most neglected domestic animals, and such unregulated free-roaming behavior can make pet dogs important spreaders of many zoonotic pathogens to their owners. Low levels of hygiene practice, a lack of proper health awareness among people, and insufficient veterinary attention contribute to the increased risk of such infections (18). Most *Dipylidium* infections are asymptomatic and self-limited, though abdominal pain, restlessness, and agitation can occur (19). Public concern over canine parasitic diseases has been aggravated by the high and uncontrolled number of stray dogs in urban areas that shed parasite eggs and oocysts, representing a source of infection for humans (20). Moreover, these pathogens can be found in standard human environments, such as water, soil, food, parks, and contamination from dog feces, and represent a high risk to immuno-suppressed persons, children, seniors, and pet owners. Thus, dogs play an important role in the transmission of parasites in public health, so it is necessary to control the dog population through various means.

### Rationale of Study

It is very much necessary to study the exact epidemiology of the dog's status in the Siddharthanagar Municipality. Dipylidiasis itself is a neglected parasitic zoonosis (21,22). This study will provide basic data on the prevalence of *D. caninum* in Siddharthanagar municipality to assess the potential risk of dipylidiasis in dogs and humans (23). Due to the potential risk of zoonotic helminths to human beings and the low level of zoonose awareness among pet owners, there is a need to generate awareness among pet owners regarding the periodic anthelmintic treatment of pet dogs and other prevention and control measures (24,25). Although dipylidiasis is rare in humans, it still poses risks to children. Research shows that among the 33 reported cases in 2000 in humans, the majority of patients, 90.9% (30/33), were children (26). This study shows that the children are at potential risk. To the best of our knowledge, this infection is scattered throughout the world, and at least 349 human cases of dipylidiasis have been reported to date, according to medical literature on human dipylidiasis (27). The hypothesis is that there is a certain number of gastrointestinal parasites present more in stray dogs in comparison to pet dogs and to assess potential risk factors that lead it to be a zoonotic threat in Rupandehi district, Nepal. Hence, this study will explore the zoonotic importance of *D. caninum* in Siddharthanagar.

### Objectives of Study

#### General Objectives

- To determine the prevalence of *D. caninum* in stray and pet dogs of Siddharthanagar municipality, Rupandehi.

#### Specific Objectives

- To diagnose the eggs of *D. caninum* in the faces of dog
- To study the various risk factors (age, sex, breed, tick infestation) associated with the infection of *D. caninum*
- To be able to know about the zoonotic and public health importance of *D. caninum*
- To aware the society through research-based data and values
- To raise awareness about zoonotic helminths among pet dog owners of Siddharthanagar municipality

#### Limitations of Study

- Limited time frame, prevalence only at a particular season
- Lack of actual dog's population census
- The unwillingness of the owner to interact with us
- Difficulty in estimating accurate age of community dogs
- Lack of human resources researching dogs
- Incomplete information from pet owners

### Literature Review

#### Epidemiology

The reported prevalence of *D. caninum* in published studies varies from 4.0% to 60.0% in dogs. Both dogs and cats are susceptible to infection with *D. caninum* by ingesting intermediate-host infected fleas (*Ctenocephalides felis*, *Ctenocephalides canis*, *Pulex irritans*) or, more rarely, lice (*Trichodectes canis*). Humans, usually young children (0.5–5 years old), probably acquire the infection after accidental ingestion of fleas or lice during contact with pets (28,29).

#### Prevalence

Out of 98 positive samples, five different parasite species were observed, including *Ancylostoma* spp. 52.0% (51/98), *Toxocara canis* 41.8% (41/98), *Taenia/Echinococcus* spp. 15/98 (15.3%), *D. caninum*, 9.2% (9/98), and *Trichuris vulpis*, 5.1% (5/98) (30). Romero (31) conducted a study in Mexico City. A total of 1603 pet feces were collected and classified according to gender and age (young animals < 1 year and adults > 1.1 years). The results showed that 13.10% (210) of the dogs were positive for some type of gastrointestinal parasite (*Toxocara* spp., *Coccidia*, *Ancylostoma* spp., *Giardia* spp., *D. caninum*), of which 115 (13.18%) were males and 95 (12.99%) females, with no gender difference ( $\chi^2 = 0.013$ ,  $P = 0.99$ ).

A study in Sudan (32) on the data distribution of the various worm species in positive dogs indicates that *Ancylostoma caninum* eggs are by far the most common (55.6%). The other detected worm egg species and their respective frequencies were: *Toxocara canis* (51.9%), *D. caninum* (50.0%), *Taeniidae* (33.3%), *Toxocara leonina* (25.9%), *Diphyllobothrium latum* (5.6%), *Spirocerca lupi* (3.7%), and *Physaloptera canis* (3.7%). Out of the total 340 stray dogs in Jordan, El-Shehabi (33) reported the overall prevalence of intestinal helminths to be 70.3%, whereas the prevalence of *D. caninum* was 19.4%. In Ethiopia, Degefu collected a fecal sample from 334 dogs where the helminth parasite infection was detected in 215 (64.4%) dogs, and the species of helminth parasites found with their relative frequencies were: *Ancylostoma caninum* (58.8%), *Toxocara canis* (25.8%), *D. caninum* (25.8%), *Taenia spp.* (18.3%), *Toxocara leonina* (16.8%), and *Trichuris vulpis* (0.6%). There was a significant difference in the overall prevalence between adult and young animals ( $P < 0.05$ ).

## Methods

### Materials Required

- Fecal sample
- Zipper plastic bags
- Gloves
- Ice packs
- Mortar pistol, stainless sieve
- Glass slides
- Compound microscope
- Fecal sample
- Centrifuge tubes
- Centrifuge tube rack
- Centrifuge machine
- Color spray

### Study Area

The sample required for research will be collected from

the community as well as owner dogs of Siddharthanagar municipality, Rupandehi district, Lumbini province, which lies at a latitude of 27.6934°N to 83.3148°E and longitude of 83.343°E and has an altitude of 110 m above sea level. The total area of this municipality is 36.03 sq. km. It borders India and Sonauli in the Maharajgunj district of Uttar Pradesh to the south and lies 25 km east of Lumbini. It is bounded by Padsari and Pharsatikar VDC to its north, Bagha and Basantapur VDC to its east, and Hati Bangai VDC to its west. This municipality has a maximum temperature of 33 °C and a minimum of 25 °C, with an average annual rainfall of 1600 mm.

### Study Design

This cross-sectional study aimed to identify the prevalence of *D. caninum*. It consists of epidemiological data collection, fecal sample collection, laboratory examination, analysis, interpretation, and result discussion. The research was started in June and ended in August 2021 in Siddhartha Municipality (Figure 1). A questionnaire survey was prepared for pet owners, which assessed their awareness of the dog's zoonotic helminth infection, vaccination, and deworming.

### Sampling Method

Purposive sampling was applied in this study.

### Variables

The independent variable is the presence of *D. caninum* eggs in a fecal sample, while the dependent variables include qualitative factors such as sex, breed, dehydration, temperature, capillary refill time (CRT), and ectoparasite infestations, along with quantitative factors like weight, age, heartbeat, respiration rate, temperature, CRT, and body condition score.

### Sample Size

The sample size was calculated online by using the

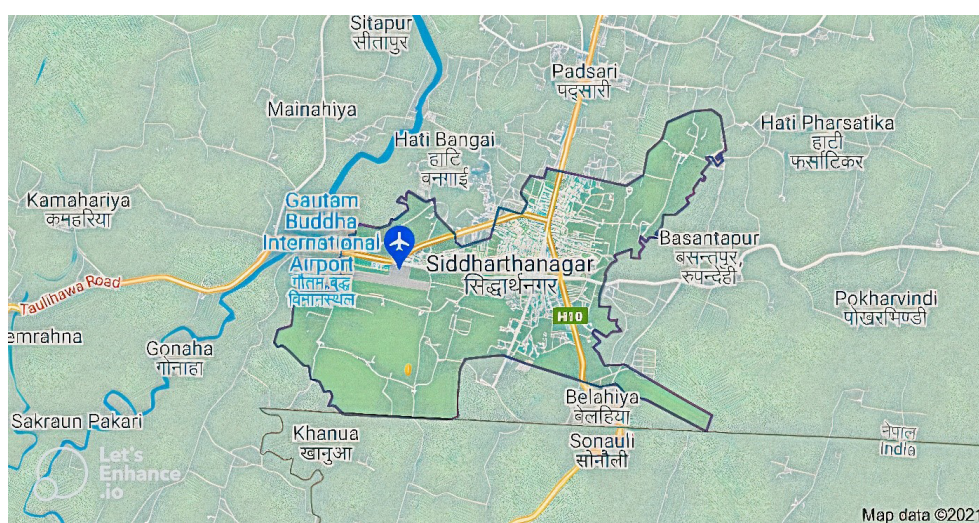


Figure 1. Map of Siddharthanagar Municipality

EpiTools epidemiological calculator given by Ausvet with a specified level of confidence and precision. The study was carried out at a 95% level of confidence with a desired precision of 0.05. The formula given by Ausvet (34) is as follows:

$$n = [Z^2P(1-P)]/e^2$$

Where, Z = standard normal distribution corresponding to desired confidence level (Z=1.96 for 95% CI); P = estimated true proportion (13.5% based on previous study); e = desired precision (0.05)

Using the above protocol, we found the required sample size to be 180 for an infinite population. On this basis, 200 fecal samples (100 communities and 100 pet) were collected purposively from different wards of Siddharthanagar municipality.

### **Epidemiological Data Collection**

Epidemiological data for this study was collected using an interview questionnaire, a structured record-keeping sheet, physical inspection, and face-to-face discussions with pet owners. The structured questionnaire obtained various data, including age, sex, breed, deworming status, body condition score, tick infestations, recent medications, feeding status, etc.

### **Sample Collection**

Samples were collected from both community and pet dogs in the early morning. Pet dog fecal samples were collected directly from freshly voided faces, and owners answered questionnaires. Community dogs were caught using catch poles, a dog's net, and a handler. Then, proper restraining was done, and a fecal sample was collected directly from the rectum. The procedure involved:

- After appropriate restraining from a dog using a muzzle, about 5 g of fecal sample was collected directly from the dog's rectum using a gloved index finger.
- The collected sample was kept in zip-lock plastic and labeled properly with dog type (pet or stray), age, sex, breed, BCS, tick infestation, and date of collection. The age of stray dogs was estimated by dentition analysis (35).
- The peri-anal region of the dog was also examined for the presence of gravid proglottids, which were observed in infected dogs (36).
- Then, the zip-lock plastic containing the collected sample was kept in a cool pack.
- Stray dogs, after the collection of samples, were marked using a non-irritant spray to prevent the repetition of samples from the same dog.
- The collected samples were transported to the lab, stored in the refrigerator at about 2-4° C, and examined on the same day.

### **Laboratory Examination**

#### **Principle**

The fecal flotation technique is most commonly used in veterinary medicine for the examination of feces, which is based on the principle that the parasite eggs are less dense than the fluid flotation medium. It is the most satisfactory method because it involves separating the eggs from fecal debris by floating them in a variety of solutions (37,38). Parasite eggs, cysts, and oocysts are concentrated on the surface of a medium because of their lighter density, and the result is a clean preparation for microscopic examination with a minimal amount of distracting fecal debris (39). Microscopic examination of fecal samples was done qualitatively by centrifugal flotation techniques as described by Foreyt and Foreyt (40). The procedure is as follows:

- About 4 g of the fecal sample was mixed with 56 ml flotation solution and ground properly using a mortar and pestle.
- Flotation solution preparation: 400 g sodium nitrate in 1000 mL of water
- The mixture was then strained with a tea strainer.
- The resultant filtrate was then centrifuged using a centrifuge machine at 1500 rpm for 5 minutes in a 15-mL centrifuge tube.
- The resultant filtrate was then stirred properly to let the eggs come up to the surface (eggs have a lower sp. gravity than flotation solution).
- Using a pipette, a small amount of flotation solution from the surface was filled in the slide and allowed to stand for 5 minutes. The slide was observed under 10X magnification for the presence of *D. caninum* eggs.
- Eggs were identified based on the characteristics of egg packets.

#### **Data Analysis**

Data were analyzed using descriptive statistical methods using MS Excel and R-Studio version 4.1.0. The effect of dog type (pet or stray), age, and sex on the prevalence was evaluated using the Chi-Square test for independent categorical variables.  $P < 0.05$  was considered significant. Finally, tables and charts were used to present the results generated by R-Studio and MS-Excel 2016.

Overall prevalence was calculated as:  $P = \text{Number of positive samples} / \text{total number of samples examined (200)}$

Confidence interval estimated around the observed prevalence was calculated as:  $CI \text{ estimate} = P \pm Z \sqrt{\{P(1-P)\}/n}$

Where, P = point of estimate or prevalence rate; Z = 1.96 for 95% CI; and n = total number of samples

#### **Ethical Consideration**

A wide range of safety measures have been adopted

during the process of sample collection for both animals and individuals. Oral consent was obtained from the pet owners before commencing fecal sampling of the pet dogs. Easier restraining and quicker activities were done to these animals to reduce the pain.

## Results

### Overall Prevalence of *Dipylidium caninum*

A total of 200 fecal samples were collected from different wards of Siddharthanagar municipality. Out of them, 25 (12.5%) were positive for the prevalence of *D. caninum* (Table 1).

### Prevalence Among Different Dog Types

Of the 100 pet and 100 stray dog fecal samples examined, the prevalence was higher in stray dogs, which showed an 18% prevalence (18/100), than in pet dogs, which showed a 7% (7/100) prevalence (Table 2). The association between dog type and prevalence was found to be statistically highly significant, with a *P* value of 0.01.

### Sex-wise Prevalence of *Dipylidium caninum*

Out of 113 male and 87 female dogs examined, the prevalence of *D. caninum* was 14.15% (16/113) and 10.34% (9/87), respectively (Table 3). The prevalence was higher in males, but the difference was not significant.

**Table 1.** The Overall Prevalence of *Dipylidium caninum*

Total Sample (N)	Positive	Negative	Prevalence	95% CI
200	25	175	12.5%	0

**Table 2.** Prevalence Rate Among Pet and Stray Dogs

Type	No. of Total Samples	No. of Positive Samples	Prevalence Rate (%)	Odds Ratio (95% CI)	Chi-square	<i>P</i> Value
Stray	100	18	18%	0.34 (0.11-0.91)	5.53	0.01
Pet	100	7	7%			

**Table 3.** Table Showing Sex-wise Prevalence

Sex	No. of Total Samples	No. of Positive Samples	Prevalence Rate (%)	Odds Ratio (95% CI)	Pearson's Chi-Square value	<i>P</i> Value
Male	113	16	14.15%	1.43 (0.56-3.87)	0.65	0.42
Female	87	9	10.34%			

**Table 4.** Age-wise Prevalence

Age Group	No. of Total Samples	No. of Positive Samples	Prevalence Rate (%)	Odds Ratio (95% CI)	Chi-Square value	<i>P</i> Value
Less than 1 y	75	6	8.0%	0.48 (0.15-0.45)	2.22	0.13
More than 1 y	125	19	15.20%			

**Table 5.** Prevalence Based on Tick Infestation

Tick Infestation	No. of Total Samples	No. of Positive Samples	Prevalence Rate (%)	Odds Ratio (95% CI)	<i>P</i> Value
Tick present	107	22	20.56%	7.89 (2.19-41.58)	0.002*
Tick absents	93	3	3.22%		

\* Statistically significant.

### Prevalence Among Different Age Groups

The total sample population was divided into two age group categories: those over one year and those under one year. The prevalence of *D. caninum* was found to be 15.20% (19/125) and 8.0% (6/75), respectively, in these age groups (Table 4). The difference in prevalence among age groups was insignificant, with a *P* value of 0.13.

### Prevalence Based on Tick Infestation

Out of the 200 samples collected, the tick infestation was 42.50% (85/200). The prevalence was 3.22% (3/93) in non-tick-infested dogs and 20.56% (22/107) in tick-infested dogs (Table 5). The difference in prevalence among the tick-infested and non-infested dogs was statistically significant, with a *P* value of 0.002.

## Discussion

The overall prevalence of *D. caninum* (Figures 2 and 3) in Siddharthanagar municipality was found to be 12.5% (Figure 4), which nearly coincides with the cross-sectional study done by Yadav and Shrestha, which found the prevalence of gastrointestinal helminth in the stray and pet dogs of Siddharthanagar municipality. We found the prevalence to be 11.5%. The prevalence of helminth parasites was significantly higher in stray dogs as compared to pet dogs (Figure 5) ( $P < 0.05$ ).

Research conducted on study on the prevalence of gastrointestinal zoonotic helminth in dogs of Kathmandu, Nepal, in a total of 210 fecal samples (105 for each pet and stray), where the prevalence of *D. caninum* was found to be 9.2% (9/98), where the prevalence was higher in stray

dogs (56.2% vs. 37.1%) ( $P < 0.05$ ), in females (51.6% vs. 39.8%) ( $P > 0.05$ ) (Figure 6), and in non-dewormed dogs (72.7% vs. 33.5%) ( $P < 0.05$ ). Similarly, only 11.4% of owners were aware of canine helminth zoonoses.



Figure 2. Gravid Proglottid of *Dipylidium caninum*



Figure 3. Egg capsule of *Dipylidium caninum*

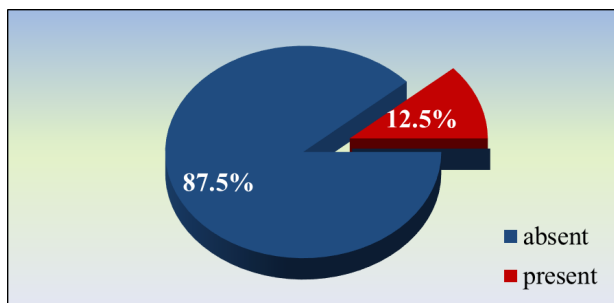


Figure 4. The Overall Prevalence of *Dipylidium caninum*

A higher prevalence was reported by Abere et al (41), where the overall prevalence of gastrointestinal helminth infection in pet and stray dogs was 75.26% and 84.78%, respectively. Similarly, the prevalence of *D. caninum* was found to be 29.75%. The sex or breed group didn't significantly affect the prevalence of the parasite. There was no significant difference ( $P > 0.05$ ) in the prevalence of GI helminth ( $P > 0.05$ ) between males and females, and there was also no significant difference between local (76.70%), exotic (33.80%), and crossbred (76.19%) pet dogs. A lower prevalence was reported (42) in the state of Enugu, south-eastern Nigeria, where the prevalence of *D. caninum* was found to be 4.0%. This study shows that the difference was statistically insignificant between sexes ( $P > 0.05$ ). The prevalence of helminth parasites has been shown to vary considerably from one geographic region to another depending on the genera of helminth involved, animal species, and local environmental conditions such as humidity, temperature, rainfall, vegetation, and management practices.

Beugnet et al (42,43) examined the prevalence of *D. caninum* in fleas collected from domestic dogs in Europe. This European survey demonstrated the transmission of *D. caninum* between fleas on dogs and cats across Europe. Out of the 2828 dog fleas collected from dogs, 3.1% were found to be infected (44) *D. caninum* infection from dog fleas (*Ctenocephalides canis*) was found almost always  $> 95\%$  in fleas collected from proglottids shed by domestic dogs. There was no significant difference between breeds in the research of *D. caninum* infection in dogs and humans in Bishoftu town, Ethiopia, where the prevalence was found to be 21% and between local and exotic breeds was 17.7% and 2.86%, respectively (45). Hence, it was concluded that the age group of dogs can be infected irrespective of age (Figure 7). The roaming nature and various factors including tick infestation (Figure 8) depend on the infection of *D. caninum* in dogs (46,47).

### Conclusion

The research study shows that the prevalence of *D.*

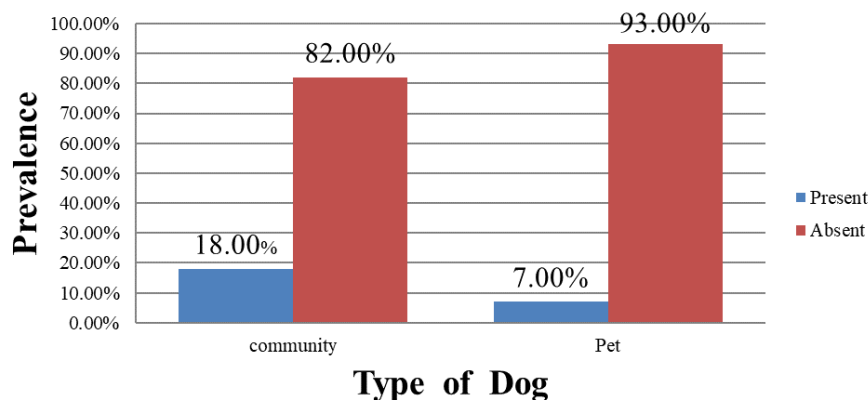


Figure 5. Prevalence Rate Among Stray and Pet Dogs

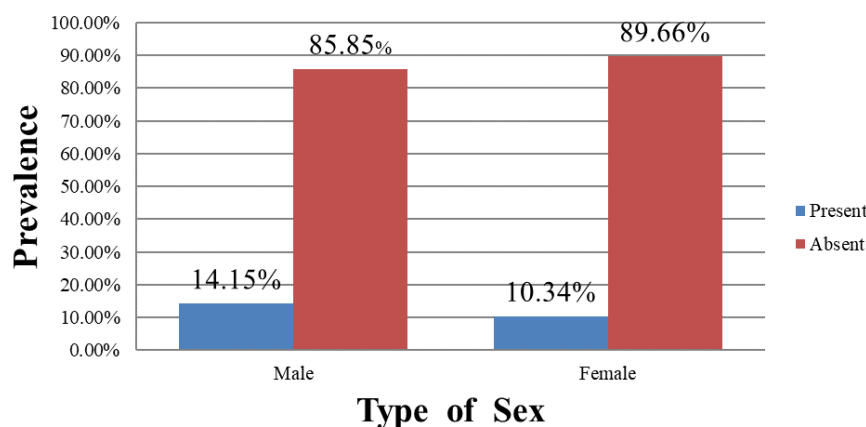


Figure 6. Bar Graph Showing Sex-wise Prevalence

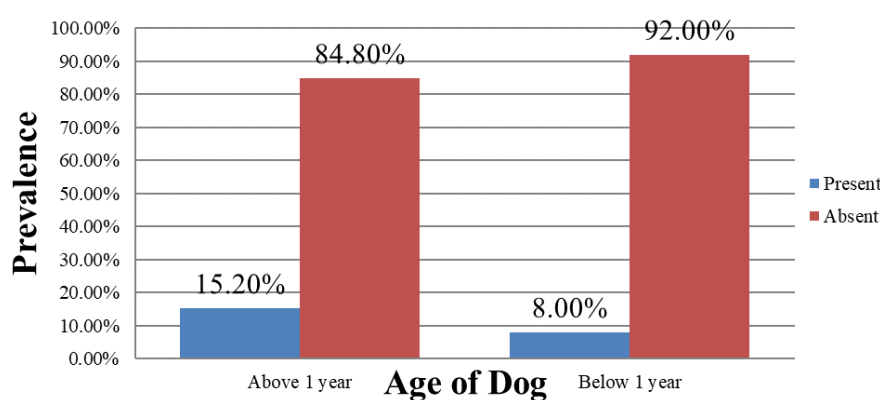


Figure 7. Bar Graph Showing Prevalence Among Different Age Groups

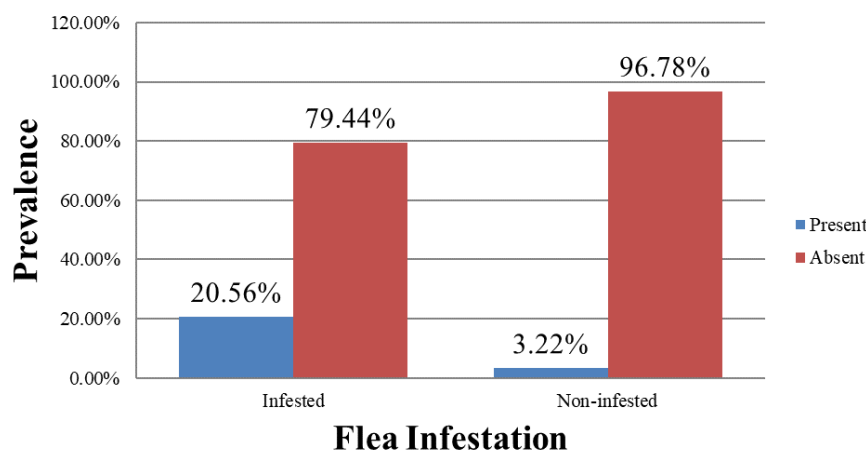


Figure 8. Prevalence Based on Tick Infestation.

*caninum* is significantly higher in stray dogs than in pet dogs. The history of deworming status and vaccination status in stray dogs was unrecorded, which might pose the risk of infection to other dogs as well as humans. There is no significant difference in age or sex between the two groups. The prevalence is higher in the local breed as compared to the crossbreed, but the significance was

statistically insignificant. Similarly, the deworming status of pet dogs is statistically insignificant. The prevalence of tick-infested dogs is higher than that of non-infested dogs, and the significance between them is also statistically significant. This suggests the regular need for anthelmintic treatment and control of the stray dog population through various animal birth control programs. Almost 13.5% of

the sampled population was aware of the canine helminth zoonoses. According to the survey taken from owners, it is necessary to increase awareness regarding deworming, regular vaccination, rearing management, and dog practices, which also helps to minimize the prevalence of GI helminths in Siddharthanagar municipality.

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#### Author's Contribution

**Conceptualization:** Rashok Khanal, Keshav Raj Malla.

**Data curation:** Keshav Raj Malla.

**Formal analysis:** Keshav Raj Malla.

**Funding acquisition:** Keshav Raj Malla, Birendra Shrestha.

**Investigation:** Keshav Raj Malla, Birendra Shrestha.

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**Project administration:** Rashok Khanal, Keshav Raj Malla, Birendra Shrestha.

**Resources:** Keshav Raj Malla.

**Software:** Rashok Khanal.

**Supervision:** Birendra Shrestha.

**Validation:** Birendra Shrestha.

**Visualization:** Rashok Khanal.

**Writing—original draft:** Rashok Khanal, Keshav Raj Malla.

**Writing—review & editing:** Rashok Khanal.

#### Competing Interests

The authors declare that there is no conflict of interest.

#### Ethical Approval

No ethical approval is needed.

#### Funding

None.

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